NTP Research Concept: Di(2-ethyl)hexyl Phthalate (DEHP) and Phthalate Mixtures.

Project Leader

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Nomination History

DEHP (and other phthalates) have been nominated on a number of occasions to the NTP for testing. In particular, many aspects of the research proposed in this document would fall under previously approved nominations for the study of peroxisome proliferators (initiated in the 1990s), the nomination of DEHP by FDA in 2004 (http://ntp.niehs.nih.gov/go/316), and the critical data needs highlighted in the NTP Center for the Evaluation of Risks to Human Reproduction monograph on DEHP issued in 2006 (http://cerhr.niehs.nih.gov/chemicals/dehp/dehp-eval.html).

Background

DEHP is a ubiquitous environmental contaminant that has been shown to produce reproductive, developmental and cancer effects in rodents. The cancer risk assessments conducted by a number of different regulatory authorities have changed over time with the advent of detailed mechanistic information on the involvement of PPAR-α (peroxisome proliferator activated receptor – alpha) in the carcinogenic process. In 1992, based on hepatocarcinogenesis in rodents (predominantly from NTP studies) the EPA and then IARC classified DEHP in category 2. Much later, a paper (Doull *et al.* 1999) proposed that the liver tumors were due to PPAR-α activation and that this mechanism was not relevant for humans and should not be used in human risk assessment. This mechanistic body of work resulted in the delisting of DEHP by IARC (IARC 2000) and the European Union (CSTEE 2004) as a potential carcinogen (i.e. category 3).

Since this time a further paper in the Sprague-Dawley rat has indicated that the liver is not the sole target for DEHP carcinogenicity in lifetime studies, with testicular as well as liver tumors also being observed (Voss *et al.* 2005). Pancreatic acinar adenomas have also been reported as treatment related findings in chronic studies in the F-344 rat (David *et al.* 2000). Moreover, a recent paper in which PPAR-α null mice were exposed to DEHP for 22 months (Ito *et al.* 2007), indicated that more liver tumors occurred in the **null mouse** than in the wild type animals. These data would imply that factors other than PPAR-α are involved in DEHP hepatocarcinogenesis, as has been suggested by others (Melnick 2002; Melnick *et al.* 2003).

When rats are exposed *in utero* to DEHP, this agent produces a range of developmental effects including lowered fetal testosterone levels, anti-androgenic phenotypes and reproductive tract malformations (Gray *et al.* 2000) in an identical manner to that observed for di-*n*-butyl phthalate (DBP) (Foster 2005, 2006). DBP produces testicular dysgenesis in rats that results in Leydig cell tumors of the testis in long term follow-up of exposed offspring after only a 10-day exposure *in utero* (Barlow *et al.* 2004).

PPAR-α has been associated with developmental toxicity produced by other agents, including perfluorooctanoic acid (PFOA). The developmental toxicity of PFOA has been examined in

PPAR-α -null mice (Abbott *et al.* 2007) and while the post-natal manifestations of PFOA (early pup death) were not seen in the null mice, the *in utero* developmental effects of PFOA were observed (embryo-fetal death). These data would imply that PPAR-α expression is developmentally regulated in the mouse. Thus, a critical issue for future risk assessments of DEHP will be the influence of exposure throughout different developmental ages (including the perinatal period) and determining the role, if any, of PPAR-α in these responses, since the information on mechanistic relevance used in the current risk assessments by IARC and the EU appears flawed. The use of DEHP as a model compound may have applicability to other phthalate esters to which we know humans are exposed and potentially other PPAR-α ligands.

The CDC has been monitoring exposure to various phthalates (including DEHP) in human urine as part of the National Health and Nutrition Examination Survey (NHANES) and has noted a high frequency of exposures to multiple phthalates in the general population (see for example (Blount *et al.* 2000; Calafat and McKee 2006; Silva *et al.* 2004a). In a much smaller study, multiple phthalate metabolites have also been measured in human amniotic fluid samples (Silva *et al.* 2004b). Such samples potentially provide the best estimates of exposure for human fetuses that could be used in direct comparison to the levels found in the amniotic fluid of rodents at dose levels that can induce reproductive tract malformations (Calafat *et al.* 2006).

An important issue in any risk assessment for phthalate esters, is what is the contribution of mixed phthalate exposures to adverse outcomes? Recent papers have indicated that the *in utero* effects of mixtures of phthalates (Howdeshell *et al.* 2007) or antiandrogens (Metzdorff *et al.* 2007) show dose additivity in response.

Hypotheses

1. That lifetime (perinatal + 2 year) exposure to DEHP would impact the dose response, incidence and/or severity for cancers of the liver and testis (and perhaps the pancreas) compared with adult only exposure.

2. That PPAR-α is developmentally regulated in the rat and unlikely to contribute to toxicity initiated *in utero* after exposure to DEHP.

3. That exposures to mixtures of phthalates, based on their individual potencies, would result in dose addition for cancer outcomes.

Proposed Research Program

1. Undertake a "perinatal" cancer bioassay with DEHP in the Wistar Han rat to address any additional contribution of early life exposure to cancer outcome after exposure *in utero*, in early life and as an adult. This would allow a more complete assessment to be made of carcinogenic potential and should allow the evaluation of targets other than the liver. The Wistar is known to respond to the effects of DEHP *in utero* (Wilson *et al.* 2007). This perinatal study should be compared to an "adult only" study in the same strain to address directly hypothesis 1.

2. Undertake an ontogeny study of PPAR-α in the Wistar Han rat. Such a study would determine when the receptor is first expressed in target tissues to complement the PPAR-α null mouse work conducted with PFOA. Since the antiandrogenic effects of DEHP (and other active phthalates) are not found in the mouse, the use of a PPAR-α null mouse approach *in utero* would not yield the toxicity information required.

3. As a second tier of study, it is proposed to undertake perinatal phthalate mixture studies using the Toxic Equivalency Factor (TEF)-type approach. Such studies should be approached with care. In particular there are a number of specific issues that require consideration:

    a. Route of exposure and associated kinetics. Choice of route of exposure would be very important (diet vs. gavage). To obtain more precision of external dose and to minimize dose intervals (there is a large variability in diet consumption during pregnancy and lactation that is not mirrored by bodyweight changes), gavage should be considered. To support these studies, toxicokinetic (TK) data and estimates of internal dose are required in the Wistar (Han) rat during pregnancy and lactation by both gavage and dietary routes.

    b. Short-term assays on a number of phthalates (e.g. di-n-butyl (DBP), di-isobutyl (DiBP), butylbenzyl (BBP), di-isononyl (DINP) and DEHP) would be required to develop potency estimates in the Wistar (Han) rat. For *in utero* exposures, the potency estimates would be via measurements of fetal testicular testosterone levels. For weanlings, some estimates of hepatic peroxisome proliferator activity would be required (e.g. CYP IVA1, Acyl CoA Oxidase etc). It is anticipated that no more than 3 phthalates would be evaluated in any long-term mixture study.

    c. Individual TK data on esters that were selected to go forward to longer-term studies would be required.

These data would guide the needs for individual perinatal bioassays and mixture work to support the DEHP study identified above. Since the question of cumulative risk for phthalates has been submitted recently to the National Academy of Sciences by EPA, this overall approach is seen as providing extra impetus to fill these data gaps.

Significance

Such studies would:

1. Provide a cancer hazard assessment for lifetime exposure to DEHP and address some of the critical questions posed with regard to the influence of early exposures on cancer outcome.

2. Elucidate the developmental ontogeny of PPAR-α in the rat and relationship to DEHP cancer (and other developmental toxicity) outcomes.

3. To provide toxicity data on important environmental phthalates during lifetime exposures (perinatal + 2 years). In addition, to provide the critical data to undertake mixture studies using the TEF approach, to inform on potential cumulative and aggregate cancer risk. Recent data have indicated that because of similar modes of action *in utero*, phthalate esters do show dose addition when administered in combination and thus it would be appropriate to consider cumulative risk for the class since human subjects (including fetuses) are typically exposed to multiple phthalates.

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